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Short communication

Inactivation of Shiga Toxin-Producing *Escherichia coli* in lean ground beef by gamma irradiation

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ABSTRACT

In this study the radiation resistance of 40 Shiga Toxin-Producing *Escherichia coli* (STEC) isolates which contained various combinations of the shiga toxin 1 (*stx1*), shiga toxin 2 (*stx2*), intimin (*eae*), and hemolysin (*ehx*) genes were determined. The STEC were suspended in lean ground beef and irradiated at 4 °C. D_{10} values, the radiation dose needed to reduce 1 log (90%) of a microorganism, ranged from 0.16 to 0.48 kGy, with a mean of 0.31 kGy for the 40 isolates. Isolates associated with illness outbreaks had a mean D_{10} of 0.27 kGy, while non-outbreak isolates had a mean D_{10} of 0.36 kGy ($p < 0.05$). The presence or absence of *stx1*, *stx2*, or both *stx*1 and 2 had no affect on D_{10} ($p > 0.05$). The presence (0.30 kGy) or absence (0.35 kGy) of *ehx* had no affect on D_{10} ($p > 0.05$). However, the mean D_{10} of isolates lacking *eae* (0.37 kGy) were significantly higher than those containing *eae* (0.27 kGy) ($p < 0.05$). There was no difference in D_{10} for isolates lacking *eae* regardless of whether or not they were associated with a foodborne illness outbreak ($p > 0.05$). It may be possible to use some of the STEC isolates which lacked *eae*, *ehx*, or both ($D_{10} > 0.30$) as avirulent surrogates in food irradiation research. The data presented in this study provides risk assessors data for metagenomic analysis as well as food and radiation processors with valuable information to control of STEC in meat.

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1. Introduction

Shiga Toxin-Producing *Escherichia coli* (STEC), is responsible for approximately 176,000 illnesses, 3700 hospitalizations, and 30 deaths in the US annually (Scallan et al., 2011; Scharff, 2010). These pathogenic bacteria are extremely diverse and more than 400 STEC serotypes have been isolated from human patients (Scheutz and Stockbine, 2005). Non-O157 STECs (serovars other than O157:H7) are now responsible for over 60% of STEC-induced illnesses (CDC, 2010a, b; EFSA, 2011) and are common contaminants in ground beef (USDA FSIS, 2014, 2011).

The resistance of STEC isolates to a variety of intervention technologies and stresses varies greatly on the intervention technology, its mode of action, food matrix, and genotype (Wang et al., 2012; Rajkowski and Bari, 2012; Enache et al., 2011; Tosa and Hirata, 1999; Benito et al., 1999; Buchanan et al., 1998). Relatively few commercialized non-thermal intervention technologies are available to inactivate pathogens in raw ground beef. Ionizing radiation (IR) is a safe, effective, and sustainable technology for improving the microbiological safety and shelf-life of foods. IR treated meat has been approved by the US Food and Drug Administration and World Health Organization approved process, and inactivates bacteria by damaging their genetic material and proteins, rendering them unable to reproduce (US FDA, 2012; Krisko and Radman, 2010; Diehl, 1995).

Recent trends in food safety lean towards the large data approach in order to understand how the genomics of a microbial community affect its interaction with the environment (National Research Council of the National Academies, 2007). In addition, the idea that pathogen virulence factors can affect resistance to food safety intervention technologies is relatively new and controversial concept (Acheson, 2014). In previous studies, the D_{10} for *E. coli* O157:H7 suspended in ground beef ranged from 0.13 to 0.34, in which single isolates or multi-isolate cocktails of serovar

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O157:H7 were used (Sommers and Fan, 2011). This data set is limited because of the small number of STEC examined, and because USDA FSIS now requires testing of meat for serovars O26, O45, O103, O111, O121, and O145 in addition to O157:H7 (USDA FSIS, 2011).

The objectives of this study were to: 1) determine the gamma radiation D_{10} of a large set of genetically diverse set of STEC inoculated in ground beef; 2) to examine the potential effect of virulence factors (eg. shiga toxin, intimin, enterohemolysin) on STEC D_{10}; and 3) identify less virulent surrogates which could be used in food irradiation research.

2. Materials and methods

2.1. Ground beef

Fresh raw ground beef (RGB, 80% lean) was purchased in-bulk from a local wholesaler. The RGB was aliquoted (90 g) into poly-nylon bags (Uline, Inc., Philadelphia, PA) and vacuum-packaged to 50 mB using a Multi-Vac A300 Vacuum-Packager (Kansas City, MO). In order to eliminate any contaminating *E. coli* the meat was gamma irradiated (−20 °C, 2 kGy) using the protocol of Thayer and Boyd (2001). The meat was then stored at −20 °C until ready for use.

2.2. Shiga Toxin-Producing Escherichia coli (STEC)

STEC were obtained from the USDA-ARS Western Regional Research Center, USDA-ARS Mid-Western Center, USDA-ARS Clay Center, the Centers for Disease Control and Prevention (Atlanta, GA), and the American Type Culture Collection (ATCC, Manassas, VA, 2012). STEC origins and genotypes including O and H antigen types, and presence or absence of virulence genes were provided by those donating the isolates (Table 1).

2.3. Propagation and inoculation

The STEC were cultured independently in 20 ml Tryptic Soy Broth without dextrose (BD-Difco, Sparks, MD) using 50 ml sterile tubes at 37 °C (150 rpm) for 18–24 h using a New Brunswick Model G34 Environmental Shaker (New Brunswick, Edison, NJ). The bacteria were then sedimented by centrifugation × 1200 g using (Hermle Model Z206A, Hermle Labortechnik, Germany) and resuspended in an equal volume of sterile 0.1% peptone water (SPW, BD-Difco). The STEC was then aliquoted (10 ml) into 90 g of thawed RGB and mixed by stomaching for 2 min on medium speed (Seward Inc, Basinstoke, UK). The samples were aliquoted (10 g) into sterile filter stomacher bags, the air expelled manually, and then stored at 4 °C for 1 h until irradiation.

2.4. Irradiation

A Lockheed Georgia Company (Marietta, GA, USA) self-contained ^{137}Cs irradiator, with a dose rate of 0.070 kGy/min was used. The temperature during irradiation (4 °C), monitored by thermocouple, was maintained by introduction of the gas phase from a liquid nitrogen source. The absorbed dose was verified using 5 mm alanine pellets that were then measured using a Bruker eScan EPR Analyzer (Bruker, Inc., Billerica, MA).

2.5. Sample dilution and plating

Approximately 90 ml of SPW was added to the sample bags containing 10 g of RGB. The samples were then mixed by stomaching for 2 min and serial dilutions performed in SPW. One ml of the diluted samples were placed on duplicate *E. coli* Petifilms. (3M, Saint Paul, MN). The Petrifilms were then stored overnight at 37 °C and scored using a calibrated 3M Petrifilm Reader. The STEC phenotype was noted. There was no difference in D_{10} when the STEC were incubated on either *E. coli* or APC Petrifilms.

2.6. D_{10} values

The mean plate counts of the treated samples (N) were divided by the average control plate counts (No) to give a survivor ratio (N/No). The $\log_{10}$ (N/No) of the ratios was then used for determination of D_{10} and other statistical analyses. D_{10} were determined by the reciprocal of the slope (Diehl, 1995).

2.7. Statistical analysis

The radiation doses for each experiment were 0, 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 kGy, with single samples being used per radiation dose. Each experiment (the D_{10} of each STEC isolate) was then conducted independently three times ($n = 3$). Statistical analysis functions of MS Excel (Microsoft Corp., Redmond, WA) were used for routine calculations (D_{10} determination), descriptive statistics, analysis of variance (ANOVA).

3. Results and discussion

In this study using 40 isolates, the STEC D_{10} ranged from 0.16 to 0.48 kGy (Table 1), which was significantly higher than those obtained in previous studies. The mean D_{10} of the isolates not associated with outbreaks were greater than those from outbreaks (0.27 vs. 0.36 kGy, $p = 0.0003$, Table 2). The isolates involved in human illness, with the exception of the O104 isolates, contained the *stx* (shiga toxin), *eae* (intimin), and *ehx* (enterohemolysin) virulence genes.

Many of the isolates not associated with illness outbreaks lacked one or more virulence genes including *eae* and/or *ehx* (Table 1). *Eae* is encoded on the locus of enterocyte effacement (LEE) and allows adhesion of the STEC to intestinal cells in addidtion to coding a type III secretion apparatus and various chaperonins (Mills et al., 2013; Garmendia et al., 2005). STEC which lack *eae* (eg. O104) may possess other enteroaggregative virulence factors that allow attachment to intestinal cells (EFSA, 2011). The *ehx* (enterohemolysin) gene encodes a protein responsible for lysis of red blood cells (Li et al., 2008). All of the STEC used in this study encoded either the *stx1*, *stx2*, or both the *stx1* and *stx2* genes, with the toxin proteins attacking the blood vessels of the glomeruli in the kidney (Obrig and Karpman, 2012).

In this study STEC lacking *eae* had a mean D_{10} significantly higher (0.37 kGy) than those with *eae* (0.27 kGy) ($p = 1.1 \times 10^{-5}$). There was no difference in D_{10} between isolates associated, or not associated, with human illness which lacked the *eae* gene (Table 2, $p = 0.68$).

The difference in D_{10} for isolates containing *ehx* (0.30 kGy) versus lacking *ehx* (0.35 kGy) were not significant (Table 2, $p = 0.07$). There was no difference in D_{10} based on the presence of *stx1* (0.29 kGy), *stx2* (0.33 kGy) or both *stx1* and *stx2* (0.30 kGy) (Table 2, $p = 0.37$).

In *E. coli*, chaperonins such as GroEL are required for response to oxidative stress Hunter and Hunter (2013). Zhou et al. (1998) found that the chaperonin/heat shock proteins DnaK and GroEL were required for efficient assembly of nucleotide excision repair complexes in *E. coli*. Caillet et al. (2008) found that gamma radiation induced the expression of DnaK, GroEL and GroES in *E. coli*. It is therefore possible that expression of chaperonins encoded on the LEE-PAI could interfere with function of *E.coli's* endogenous chaperonins in response to oxidative stressors such as gamma radiation.